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Effect of *Echinococcus multilocularis* on the origin of acetyl-CoA entering the tricarboxylic acid cycle in host liver

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Abstract

Carbon-13 nuclear magnetic resonance (NMR) spectroscopy was employed to investigate alterations in hepatic carbohydrate metabolism in *Meriones unguiculatus* infected with *Echinococcus multilocularis*. Following portal vein injections of an equimolar mixture of [1,2-$^{13}C_2$]acetate and [3-^{13}C]lactate, perchloric acid extracts of the livers were prepared and NMR spectra obtained. Isotopomer analysis using glutamate resonances in these spectra showed that the relative contributions of endogenous and exogenous substrates to the acetyl-CoA entering the tricarboxylic acid cycle differed significantly between infected and control groups. The mole fraction of acetyl-CoA that was derived from endogenous, unlabelled sources (F_U) was 0.50 ± 0.10 in controls compared to 0.34 ± 0.04 in infected animals. However, the fraction of acetyl-CoA derived from [3-^{13}C]lactate (F_{LL}) was larger in livers of infected animals than those from controls with values of 0.27 ± 0.04 and 0.18 ± 0.04, respectively. Similarly, the fraction of acetyl-CoA derived from [1,2-$^{13}C_2$]acetate (F_{LA}) was larger in livers of infected animals compared to those in controls; the fractions were 0.38 ± 0.01 and 0.32 ± 0.07, respectively. The ratio of F_{LA}:F_{LL} was significantly smaller in the infected group with a value of 1.42 ± 0.18 compared to 1.74 ± 0.09 for the controls. These results indicate that alveolar hydatid disease has a pronounced effect on the partitioning of substrates within the pathways of carbohydrate metabolism in the host liver.

Introduction

Investigation of metabolic interaction between the metacestode stage of *Echinococcus multilocularis* and its host *Meriones unguiculatus* revealed that the parasite depletes hepatic glycogen and induces a persistent hypoglycaemic condition in its mammalian host (Novak *et al.*, 1989, 1993, 1995; Modha *et al.*, 1997). As maintenance of normal levels of glucose in the mammalian body is of homeostatic importance, hepatocytes are stimulated to release free glucose into the blood during periods of hypoglycaemia (Hers, 1990). This glucose is derived from the breakdown of glycogen (glycogenolysis) or from *de novo* synthesis (gluconeogenesis) from lactate, alanine and other substrates (Van den Burghe, 1991; Seifter & Englard, 1994). Evidence for an increase in the rate of host gluconeogenesis in the *E. multilocularis*–*M. unguiculatus* system was provided by Schoen *et al.* (1999) in a carbon-13 decoupled ^{1}H spin echo nuclear magnetic resonance (NMR) study of [2-^{13}C]acetate metabolism in the liver of the infected host. Also, the results confirmed that label from the exogenous acetate, through randomization of the ^{13}C in biochemical pathways of host liver, ends up in hepatic glucose and, more importantly for the study of host–parasite interaction, demonstrated that some of this newly synthesized glucose is taken up and utilized by the parasite.

In the present study we followed up on the above information and investigated whether substrate competition in the feeder pathways of the tricarboxylic acid (TCA) cycle, occurring within host hepatocytes, is altered

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by this parasitic infection, with the focus being the origin of the acetyl-CoA that enters the cycle at the citrate synthase step. This was accomplished by introducing [3-^{13}C]lactate and [1,2-$^{13}C_2$]acetate, as a mixture, into the liver, followed by quantitative analysis on the resultant glutamate isotopomers. The work of Malloy *et al.* (1990) and Sherry *et al.* (1992), which employ this combination of labelled substrates and make use of carbon-13 NMR resonances of glutamate, was made use of in performing this analysis and relating the data back to the fraction of acetyl-CoA derived from each of the labelled substrates and endogenous, unlabelled metabolites. (Glutamate is used for the analysis as it is present at higher concentration than its precursors.) Coupling is one of the most important phenomena in isotopomer analysis by NMR. In proton-decoupled ^{13}C NMR, a molecule having only one ^{13}C atom will produce a spectrum containing only one peak. However, if a ^{13}C atom in a molecule has another ^{13}C located adjacent to it, its resonance will be split and appear as a doublet in the spectrum; the separation between the two peaks in the doublet is known as the coupling constant, J, and is given in Hertz. If a ^{13}C atom has two ^{13}C atoms located adjacent to it, the doublet caused by the presence of one ^{13}C is split again resulting in a doublet of doublets or, if the J values are equivalent, a triplet. Thus, when the sample under investigation contains a mixture of isotopomers of a particular compound, the signal originating from just one carbon in the compound can result in a complex multiplet and, correspondingly, the signals resulting from all the various carbon atoms in the compound can result in a series of complex multiplets. Analysis of such multiplets in the glutamate spectra of the current study permits calculation of the fraction of the acetyl-CoA due to each of the three acetyl-CoA isotopomers formed: unlabelled acetyl-CoA from endogenous sources, [2-^{13}C]acetyl-CoA from [3-^{13}C]lactate and [1,2-$^{13}C_2$]acetyl-CoA from [1,2-$^{13}C_2$]acetate. For a detailed discussion of this analysis see Malloy *et al.* (1990) and Sherry *et al.* (1992).

The specific aims of this study were to determine how *E. multilocularis* infection influences the relative contributions of endogenous and exogenous substrates for the acetyl-CoA utilized in the citrate synthase step in the liver and, within the exogenous substrates, the relative amounts of acetyl-CoA formed by acetyl-CoA synthetase and the pyruvate dehydrogenase complex. This, in turn, provides insight into the changes in hepatic metabolism that occur as a result of an alveolar hydatidosis infection.

Materials and methods

Infection and tissue collection

Two groups of 21 *M. unguiculatus* males (6 months old) were used. All animals were cared for and utilized in accordance with the principles of the Canadian Council on Animal Care as stated in *Guide to the Care and Use of Experimental Animals*, and were allowed to feed on commercial pellets and water *ad libitum*. Each animal of one group was infected with *E. multilocularis* (Alaskan strain) by an intraperitoneal injection of 0.5 ml of cyst cell suspension, whereas animals of the other group remained uninfected and served as controls. Between days 28 and 33 post-infection (p.i.) the following procedure was applied to each animal. After anaesthetization with an intramuscular injection of sodium pentobarbitol (60 mg kg^{-1}) the abdomen was opened and 0.1 ml of a solution containing a mixture of [1,2-$^{13}C_2$]acetate (3.5 mol l^{-1}) (99 atom % ^{13}C, MSD Isotopes, Montreal, Quebec) and [3-^{13}C]lactate (3.5 mol l^{-1}) (99.5 atom % ^{13}C, Isotec Inc., Miamisburg, Ohio) was injected into the hepatic portal vein over a period of 4 min. In the case of infected jirds, livers were checked to be certain they were completely free of parasitic invasion. All animals were left to metabolize the labelled compounds for a period of 1 h, after which time the livers were excized. The organs were rinsed in sterile saline, gallbladders were removed and the liver tissue was frozen in liquid N_2 and weighed. All organs were stored at −70°C until preparation of the perchloric acid (PCA) extracts. Parasite cysts were also removed and weighed in order to determine the degree of infection. All jirds were dissected between 9:00 a.m. and 12 noon to reduce effects of temporal differences in metabolism.

Preparation of PCA extracts and NMR samples

Each liver was crushed in liquid N_2 using a precooled mortar and pestle. The ground sample was then transferred into a 50 ml homogenizing tube with 4 ml of cold 0.5 M PCA added per gram of tissue. The sample was homogenized while surrounded by an ice bath, and the resulting tissue suspension was centrifuged at 27 100 *g* for 10 min at −2°C. The supernatant was decanted into a 30 ml beaker, neutralized using KOH solution, and centrifuged again under the aforementioned conditions to remove any precipitated potassium perchlorate. At this point in the procedure, the 21 samples from each group were reduced to seven samples from each group by combining so that each of the final NMR samples would contain metabolites derived from three livers of the same group to make the samples sufficiently concentrated for ^{13}C NMR analysis. These samples were then stored at −70°C until required. The frozen samples were lyophilized to dryness and prepared for ^{13}C NMR analysis by resuspension in 2.8 ml deuterium oxide (D_2O) (99.9 atom % D, CDN Isotopes, Pointe-Claire, Quebec) and 0.2 ml of a solution containing sodium [2,2,3,3-$^2H_4$]-3-trimethylsilylpropionate (TSP) (MSD Isotopes, Montreal, Quebec) in D_2O (0.045 g ml^{-1}); the TSP served as a chemical shift standard. In addition, 0.15 g of ethylenediaminetetraacetate (EDTA) (J.T. Baker Chemical Co., Phillipsburg, New Jersey) was added to each sample in order to chelate paramagnetic ions that can lead to line broadening in NMR spectra. Samples were stirred for a minimum of 2 h, and the pH meter reading adjusted to 8.0–8.3 using sodium deuteroxide (NaOD) (99 atom % D, Aldrich Chemical Co., Milwaukee, Wisconsin) in D_2O. Following refrigeration for 1 h, each sample was centrifuged for 30 min at 119 000 *g* and 4°C. The supernatant was then transferred to a 10 mm NMR tube for ^{13}C NMR analysis.

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NMR spectroscopy and data analysis

Proton decoupled ^{13}C NMR spectra were acquired at a temperature of 27°C using a Bruker AMX-500 NMR spectrometer operating at 125.77 MHZ for this nucleus. Proton decoupling was achieved using the WALTZ-16 decoupling routine with the decoupler power set to 13 dB during the acquisition time and 20 dB for the relaxation delay. A spectral width of 26315.79 Hz, 64 K data points and a recycle time of 11.25 s were used for data accumulation; zero-filling to 128 K data points was done prior to Fourier transformation. The flip angle was 90°, the acquisition time was 1.2452 s and each sample was scanned 8000 times. In addition, data accumulation was preceded by 32 dummy scans to reduce any temperature gradients in the sample that could affect spectral resolution. Chemical shift values were assigned relative to the TSP resonance at 0.00 ppm, and peak assignments were based on published data (Malloy *et al.*, 1990) and spectra of authentic compounds. Quantification of the various glutamate (Glu) isotopomers was achieved by measuring the component peaks in the Glu C3 and C4 resonances, at 29.8 ppm and 36.3 ppm respectively. The relative contributions of exogenous [1,2-$^{13}C_2$]acetate and [3-^{13}C]lactate to the acetyl-CoA used in the hepatic citrate synthase reactions were determined through use of the following equations adapted from earlier work (Malloy *et al.*, 1990; Sherry *et al.*, 1992):

1. Mole fraction of the acetyl-CoA derived from [3-^{13}C]lactate (i.e. from labelled lactate) = F_{LL} = (A4S/A4D45)(A4Q/C3)
2. Mole fraction of the acetyl-CoA derived from [1,2-$^{13}C_2$]acetate (i.e. from labelled acetate) = F_{LA} = A4Q/C3
3. Mole fraction of the acetyl-CoA derived from endogenous sources (i.e. from unlabelled sources) = $F_U = 1-(F_{LL}+F_{LA})$

where C4 is the total area of the multiplet due to carbon 4 of Glu; C3 is the total area of the multiplet due to carbon 3 of Glu; A4S is the area of the singlet in the Glu carbon 4 multiplet; A4D45 is the area of the doublet due to the coupling between carbons 4 and 5 (i.e. $J_{C4,C5}$) in the Glu carbon 4 multiplet; C4Q is the area of the doublet of doublets due to $J_{C3,C4}$ and $J_{C4,C5}$ in the Glu carbon 4 multiplet. For a spectrum with all the above components of the Glu carbon 4 multiplet clearly labelled see Sherry *et al.* (1992).

Data were analysed statistically using an analysis of variance (ANOVA). A value of $\alpha \leq 0.05$ was deemed significant.

Results

Body and liver masses for control and infected animals are presented in table 1 together with parasite cyst masses. The body mass of infected animals was determined by subtracting the cyst mass from the total jird mass. The average masses of infected animals and their livers were significantly lower than for controls. However, macroscopic observation revealed that infected animals still had, at the time of metabolizing the labelled compounds, substantial adipose tissue throughout the peritoneal cavity, around the kidneys and intestine, and subcutaneously.

Table 1. Body, liver and *Echinococcus multilocularis* cyst masses.

Observation	Controls (n = 21)	Infected (n = 21)
Body mass (g)	72.4±6.5	65.6±6.3*
Liver mass (g)	3.27±0.46	2.99±0.33*
Cyst mass (g)	–	8.76±2.52

Body masses of infected jirds have been corrected for the cyst mass. Values given are mean±S.D.
*Statistically significant difference ($\alpha \leq 0.05$).

Results from the Glu isotopomer analysis of spectra of PCA extracts of livers from control and infected animals are presented in table 2. Spectra analogous to those obtained in this study can by found in Malloy *et al.* (1990) and Sherry *et al.* (1992). The fraction of hepatic acetyl-CoA derived from endogenous, unlabelled sources (F_U) was higher in controls than in infected animals, but the livers of infected animals contained larger fractions of acetyl-CoA derived from both [3-^{13}C]lactate (F_{LL}) and [1,2-$^{13}C_2$]acetate (F_{LA}). The ratio of F_{LA}:F_{LL} was significantly lower in infected animals.

Discussion

The results clearly demonstrate that alveolar hydatidosis has a profound effect on the partitioning of substrates within the hepatic biochemical pathways of the host. In infected animals exogenous, labelled substrates were a greater fraction of the substances serving as precursors for the acetyl-CoA destined for the citrate synthase reaction and, in both control and infected groups, [1,2-$^{13}C_2$]acetate predominated over [3-^{13}C]lactate as a precursor for this acetyl-CoA. The latter result was reaffirmed by measured values of F_{LA}/F_{LL}; these values also indicate that the preference for acetate over lactate is less pronounced in livers from infected animals.

As a prelude to a discussion of the data on acetyl-CoA isotopomers it is important to consider the ramifications of the data of table 1. Our previous work indicates that *E. multilocularis* exerts a systemic starvation effect within

Table 2. Results of isotopomer analysis using NMR spectra of PCA extracts of livers from control and infected jirds following introduction of [3-^{13}C]lactate and [1,2-$^{13}C_2$]acetate.

Determination†	Controls (n = 7)	Infected (n = 7)
F_U	0.50±0.10	0.34±0.04*
F_{LA}	0.32±0.07	0.38±0.01*
F_{LL}	0.18±0.04	0.27±0.04*
F_{LA}/F_{LL}	1.74±0.09	1.42±0.18*

*Statistically significant difference ($\alpha \leq 0.05$).
†F_{LA}, F_{LL}, F_{LA}/F_{LL} are the result of spectral measurements, whereas F_U is calculated by $F_U = 1 - (F_{LA} + F_{LL})$. F indicates mole fraction and the adjacent subscripts are abbreviations which reflect the isotopomer source; LA, LL and U indicate labelled acetate ([1,2-$^{13}C_2$]acetate), labelled lactate ([3-^{13}C]lactate) and unlabelled, respectively. Values given are mean±S.D.

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the host (Novak *et al.*, 1989, 1993, 1995). That starvation was parasite-induced in the present experiment is suggested by significant changes that occurred in body and liver masses of infected animals. On average, the total body weight of infected jirds was 9.4% less than that of controls. Livers from infected jirds weighed, on average, 8.3% less than those from controls. Both total body and liver masses were also reported to decrease in Syrian hamsters (a species of the same family as *M. unguiculatus*) that were deprived of food over a period of 4.5 days (Hegarty & Kim, 1981), and in rats after only 2 days of complete starvation (Goodman & Ruderman, 1980). These studies demonstrate a correlation between body and liver mass and nutrient deprivation, and thus support the view that the infected jirds in the current study were indeed also suffering from nutrient deprivation. The presence of adipose tissue in jirds infected with *E. multilocularis* suggests that these animals were not suffering from severe starvation, but rather a state of semi-starvation in which gluconeogenesis was still occurring.

In the metabolic reactions critical to this study, pyruvate plays a pivotal role being at the branch point for two of these reactions, i.e. its conversion to acetyl-CoA and to oxaloacetate. The latter compound, in turn, is utilized in gluconeogenesis and the TCA cycle. There are two factors that would tend to produce a lower concentration of cytosolic pyruvate in infected animals. First, the rate of hepatic glycolysis is inhibited under conditions of nutrient deprivation with a concomitant stimulation of gluconeogenesis (Hellerstein & Munro, 1994; Seifter & Englard, 1994). As pyruvate is produced by glycolysis and consumed by gluconeogenesis, parasite-induced starvation of the host should result in a depletion of hepatocellular pyruvate. That this is likely to have happened is further substantiated by evidence from previous research that gluconeogenesis is accelerated in hepatocytes of *E. multilocularis*-infected jirds (Schoen *et al.*, 1999). The second reason why the cytosolic concentration of pyruvate is expected to be lower in infected animals has to do with the synthesis of ketone bodies. Ketogenesis is known to increase in livers of starving mammals (Seifter & Englard, 1994) and, more specifically, Schoen *et al.* (1999) have provided evidence for accelerated ketogenesis in *E. multilocularis*-infected jirds. One of the ketone bodies, acetoacetate, is transported out of the mitochondrion in exchange for pyruvate (Kummel, 1987; Morand *et al.*, 1993). Increased production of ketone bodies and the need for these compounds in extra-hepatic tissues would result in an increase in the rate of export of acetoacetate out of hepatic mitochondria. Such an increase in rate would result in a depletion of cytosolic concentrations of pyruvate as a molecule of pyruvate must be imported for every molecule of acetoacetate exported.

Given that lactate dehydrogenase (LDH) is regulated by the intracellular ratio of pyruvate/lactate, a lower concentration of pyruvate in infected animals will facilitate the conversion of lactate to pyruvate. This argument in favour of a more rapid rate of lactate utilization in the current study is supported by the work of Morand *et al.* (1993) which reports such an increase in hepatocytes isolated from rats starved for 24 h. An increase in the conversion of lactate to pyruvate relative to the situation in control animals, given the abundance of labelled lactate, will result in a greater fraction of pyruvate that is due to the [3-^{13}C]pyruvate isotopomer and consequently a larger value for F_{LL} as pyruvate becomes converted to acetyl-CoA by the pyruvate dehydrogenase complex.

Other factors that could contribute to a larger value for F_{LL} in infected animals follow. The [3-^{13}C]lactate must first pass into the hepatocyte for conversion to pyruvate. The transport of lactate across the plasma membrane of hepatocytes is accomplished with the aid of a monocarboxylate transporter that carries either lactate or pyruvate in conjunction with a proton (Poole & Halestrap, 1993; Jackson & Halestrap, 1996). When the effects of pH on this carrier-mediated transport mechanism were studied, it was determined that the rate of lactate uptake by hepatocytes increased in a linear fashion as the extracellular pH was decreased (Fafournoux *et al.*, 1985). Owing to the excretion of acidic waste products by the parasite, it seems reasonable to assume that the extracellular environment of hepatocytes in the infected jirds is acidic, as has been observed for the liver of mice infected with another tapeworm (Blackburn *et al.*, 1993). Moreover, Metcalfe *et al.* (1988) examined the effect of starvation on the carrier-mediated transport of lactate into isolated hepatocytes and found that uptake was enhanced in cells obtained from rats starved for 48 h. Thus, because of one or both of the above effects, the transport of labelled lactate into hepatocytes may very well be augmented in animals infected with *E. multilocularis*, which would tend to increase the labelled pyruvate content and subsequently F_{LL}.

Acetate can freely diffuse across plasma membranes, and once in the cell, acetyl-CoA is readily formed by acetyl-CoA synthetase both in the cytosol and mitochondria (Crabtree *et al.*, 1990; Poole & Halestrap, 1993). Snoswell *et al.* (1982) determined that the rate of uptake of exogenous acetate by perfused rat liver is directly correlated with the concentration of acetate within the perfusion medium. Therefore, it can be assumed that under the conditions of the present experiment, the intracellular concentration of [1,2-$^{13}C_2$] acetate was high. Previous research has shown that when labelled acetate was provided to hepatocytes isolated from unstarved animals, the bulk of the label ended up in fatty acids, cholesterol and glycerol (Rabkin & Blum, 1985; Baranyai & Blum, 1989). In the present experiment, the animals were not starved prior to injection of labelled substrates, so it is expected that in livers of uninfected animals, a large proportion of the [1,2-$^{13}C_2$]acetate would have been incorporated into lipids. However, under conditions of nutrient deprivation lipogenesis is suppressed (Hellerstein & Munro, 1994), therefore utilization of labelled acetyl-CoA for lipid biosynthesis should have been very low. Thus, a decreased rate of lipogenesis might be one of the factors contributing to the observed increase in F_{LA} in infected animals.

Several research groups have investigated the phenomenon of substrate selection, and there is evidence that a type of 'metabolic channelling' may exist within the mitochondrial acetyl-CoA pool. Des Rosiers *et al.* (1991) have presented results from an experiment in

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which livers from starved rats were perfused with [2-^{13}C]acetate, and it was found that more label was detected in citrate than in ketone bodies. They theorized that acetyl-CoA produced from acetate or pyruvate may be preferentially used for citrate synthesis, while the acetyl-CoA derived from the β-oxidation of fatty acids is directed towards ketogenesis. Evidence for a similar heterogeneity in the cytosolic pool of acetyl-CoA was presented by Zhang *et al.* (1994) who examined the acetylation pattern of various drugs within the liver following the administration of various ^{13}C-labelled substrates. It was found that the mole % ^{13}C enrichment of acetate, β-hydroxybutyrate and the three acetylated drugs varied depending on the identity of the exogenous labelled compound used, be it [2-^{13}C]acetate, [1-^{13}C]octanoate or [1,2,3,4-$^{13}C_4$]docosanoate (the last two compounds are fatty acids that are metabolized in mitochondria and peroxisomes, respectively). They concluded that the extra-mitochondrial acetyl-CoA pool was not isotopically homogenous and that different sub-pools of acetyl-CoA are likely to exist within the cytosol of hepatocytes. Further, they proposed that the molecules of the various sub-pools can stream past each other without fully mixing, thus maintaining their individual fates of fatty acid synthesis, cholesterol synthesis or drug acetylation. Therefore, if metabolic channelling is indeed a significant factor in hepatocytes, it could be important in the present experiment. Normally in hepatocytes of nutrient-deprived mammals, the major source of acetyl-CoA is the β oxidation of fatty acids (Hellerstein & Munro, 1994; Seifter & Englard, 1994); this should therefore also be the most important source of unlabelled acetyl-CoA in *E. multilocularis*-infected jirds. However, if this acetyl-CoA of fatty acid origin is preferentially directed towards ketogenesis rather than citrate synthesis, there will be a greater opportunity for labelled acetyl-CoA, derived from [3-^{13}C]pyruvate and [1,2-$^{13}C_2$]acetate, to participate in citrate formation. By comparison, pyruvate generated by glycolysis is the major source of acetyl-CoA for the TCA cycle in fed normal animals (Seifter & Englard, 1994); it follows that pyruvate from glycolysis would be the most important source of unlabelled acetyl-CoA for the citrate synthase reaction in the control jirds of the current study. Therefore, if the theory of metabolic channelling (Des Rosiers *et al.*, 1991; Zhang *et al.*, 1994) is sound, with the major supply of unlabelled acetyl-CoA directed towards ketogenesis in infected jirds and towards citrate synthesis in controls, F_U should be smaller in the former animals. Of course, if F_U is decreased there will be corresponding increases in F_{LL} and F_{LA}.

In both uninfected and infected jirds, the ratio of F_{LA}:F_{LL} was greater than one, indicating that labelled acetate makes a greater contribution to acetyl-CoA than labelled lactate. A similar observation was made by Malloy *et al.* (1990) when rat hearts were perfused with a mixture of [1,2-$^{13}C_2$]acetate and [3-^{13}C]lactate. As discussed above, acetate freely diffuses across plasma membranes and is readily converted to acetyl-CoA, as the sole product, by acetyl-CoA synthetase. On the other hand, lactate, after conversion to pyruvate, forms oxaloacetate in addition to acetyl-CoA and thus it is not surprising that it contributes less to the acetyl-CoA pool. Further, the rapid build up of acetyl-CoA from acetate could impede the conversion of pyruvate to acetyl-CoA by the pyruvate dehydrogenase complex as this enzyme is inhibited by high concentrations of this product. In theory, the decrease in the ratio F_{LA}/F_{LL} in infected animals could have been brought about by relatively less [1,2-$^{13}C_2$]acetyl-CoA and/or relatively more [2-^{13}C]acetyl-CoA entering the TCA cycle. Inspection of the data in table 2 clearly shows that it is the latter that is the dominant factor. In fact, both F_{LA} and F_{LL} have increased, the reason for the observed decrease in the ratio being that F_{LL} has increased by 50% whereas F_{LA} has only increased by 19%. This indicates that *E. multilocularis* infection has influenced the pathway between exogenous lactate and mitochondrial acetyl-CoA more than that between exogenous acetate and mitochondrial acetyl-CoA, suggesting that of the factors discussed above as reasons for changes in F_{LA} and F_{LL}, those effecting the latter were more influential.

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